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Physicochemical stability and compatibility testing of Levetiracetam in all-in-one parenteral nutrition admixtures in daily practice

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Abstract

Background: Parenteral antiepileptic drugs are frequently used in critically ill patients for seizure control therapy or prevention. Many of these patients require additional parenteral nutrition (PN). Therefore, a parallel infusion of the frequently used antiepileptic drug levetiracetam (LEV) is interesting in terms of the restricted i.v. lines (e.g., neonates). The potential interactions of the complex PN admixture with the drug product and the appropriate admixing of a drug at effective dosages require physicochemical lab assessments to obtain specific and reliable pharmaceutical documentation for the intended admixing.

Aim: To assess the of compatibility and stability of LEV, a neutral and hydrophilic drug, in commercial all-in-one (AiO) PN admixtures using simple validated tests to provide necessary data in a timely manner and to allow convenient, documented and safe treatment with PN as the drug vehicle.

Methods: Different concentrations of LEV were injected into two different AiO PN admixtures with no further additives. Stability and compatibility tests for the drug and the PN admixtures were performed over seven days at +4 °C, +23 ± 1 °C and +37 °C without light protection. Stability and sample characteristics were observed by visual inspection and the validated light microscope method. Moreover, the pH level of the admixture was checked, as were the concentrations of LEV over time in the PN admixtures, using an established LC-MS/MS method.

Results: The stability controls of LEV at different temperatures were within absolute ± 20% of the theoretical value in a concentration range of 98.91-117.84% of the initial value. No changes in pH occurred (5.55 ± 0.04) and no microscopic out of specification data or visual changes were observed. The mean value of the largest lipid droplet in each visual field over seven days was 2.4 ± 0.08 µm, comparable to that of the drug-free AiO admixture. Samples stored at +37 °C showed yellowish discolorations after 96 hours of storage.

Conclusion: LEV showed compatibility and stability over seven days in the selected PN admixtures, and the described methods represented a valuable and timely approach to determine the stability and compatibility of the highly

hydrophilic, not dissociated LEV in AiO admixtures under conditions of use. Further studies with clinically relevant and representative examples of physicochemically different drug classes are needed.

Introduction

Most hospitalized patients, especially critically ill patients requiring parenteral nutrition (PN) require additional intravenous (i.v.) medications. To prevent or to treat malnutrition, PN is necessary when the gastrointestinal feeding is inefficient, nonfunctional or not possible [1]. All-in-one (AiO) admixtures represent the standard for PN regimen and include an oil/water (o/w) emulsion containing approximately 50 individual and potentially reactive soluble components. Therefore, the potential physicochemical interactions among the components, the container and admixes, such as electrolytes, vitamins, trace elements to individualize the regimen, or even i.v. medications, are numerous. The formulation is too complex to be evaluated bibliographically. Accordingly, a lab analysis of the specific composition is needed. In principal, AiO PN admixtures do not represent suited vehicles to carry drugs, but in certain situations they may be convenient or otherwise beneficial (e.g., with limited separate i.v. access, as in neonates, or for compliance, in the home PN setting). Co-administering i.v. medications via Y-site together with PN or as added to AiO PN admixture, without documented compatibility/stability as a prerequisite for both drug and PN efficacy and safety is deemed unacceptable and represents an avoidable medication error. At a minimum, short-term physicochemical compatibility and stability data are required to guarantee quality and tolerance of such an individual pharmaceutical formulation. In current nutrition practice commercial multichamber AiO PN admixtures are used at least in the short term. Documented stability of such regimens are mandatory [2]. The most common instabilities from the o/w emulsion are creaming and coalescence; physicochemical reactions may be visible by colour or pH changes and by the formation of precipitates as a result of the degradation of components or reactions between nutrients and/or drugs. These reactions depend on various factors, including concentration, light exposure, temperature, catalyst actions, trace elements, or other components, such as electronically charged ions (e.g., dissociated drugs, electrolytes) [3]. This can also lead to major, even fatal complications, such as a venous catheter occlusion or blood vessel obstruction due to precipitates or enlarged lipid droplets (> 5µm) or deposits [4-6]. The United States Pharmacopeia requires that the

mean droplet size (MDS) of a parenteral lipid emulsion has to be < 5 µm and the volume-weighted percent of fat globules ≥ 5 µm ($PFAT_5$) has to be < 0.05% (globule size distribution) [7]. To assess lipid emulsion deteriorations, simple analytical methods are mandatory to be used in a timely, reliable and cost-effective manner in daily (pharmaceutical) hospital practice to ensure the stability or compatibility of ready-to-use individual AiO PN admixtures.

There are many studies on the compatibility and stability of different medications that are admixed in PN [8-11]. However, most of these investigations are older (>10 years) and are done with analytical methods and techniques, not more state of the art. Furthermore, the composition of ready-to-use PN admixtures and the specific i.v. drug formulations have not been tested or have changed over time, and they may differ between manufacturers (e.g., generic drugs or individually manufactured, non- commercial hospital products). Therefore, it is very important to evaluate and document individual admixtures to achieve the best impact on medication practice and to avoid medication errors, especially when evaluating drugs that are frequently used in patients with PN.

Antiepileptic drugs such as levetiracetam (LEV) i.v. are frequently used in critically ill patients for seizure control therapy or prevention and treatment of a status epilepticus (e.g., in patients after traumatic or surgical brain injury, cancer patients or neonates) [12]. Such patients mostly receive multiple lifesaving and potent parenteral medications and often require simultaneous administration with PN. LEV is a new-generation antiepileptic drug that is used with a larger therapeutic range with linear kinetics, in contrast to older antiepileptic drugs. There is a lack of published clinical data on the comparative efficacy of LEV given separately or admixed to a daily PN portion. In general, there is also limited literature about PN as a drug carrier for LEV or other antiepileptics.

The aim of the present study was to evaluate the physicochemical compatibility and stability of LEV, which is an almost neutral and hydrophilic drug (see Fig. 1), admixed to commercialized PN AiO admixtures under practice conditions. In addition, suitable tests for timely assessment were proposed. Data are necessary to document this treatment framework because the possibility using of PN as a

drug carrier presents a new formulation with associated individual pharmaceutical quality responsibility.

Material and Methods

The investigations were done from January to August 2015 in the laboratory medicine unit of the Cantonal Hospital (Aarau). The LEV injection solution of 500 mg/5 mL (Levetiracetam Sandoz® concentrate for infusion; active pharmaceutical ingredient: Levetiracetam 100 mg/mL (Figure 1) [13], excipients: sodium acetate trihydrate, sodium chloride, water for injections for 1 mL) was obtained from Sandoz Pharmaceuticals AG, (Rotkreuz, Switzerland). Water, acetonitrile (ACN) and methanol (MeOH) were of LC-MS/MS grade and obtained from Sigma (Buchs, Switzerland). All other chemicals used were from Sigma (Buchs, Switzerland) and of analytical grade or otherwise indicated. Nutriflex® Lipid Special [NLS], 625 mL (lot:0333 F1) and Nutriflex® Omega Special [NOS], 625 ml (lot:0679 F1) (B Braun, Medical AG, Sempach, Switzerland), were used as PN admixtures (three-chamber-bags with separate lipid, glucose and amino acids compartments, Table 1). Three different LEV concentrations were used, representing a commonly adapted dosage range (20 mg/kg body weight) added to current PN regimens/volumes yielding LEV concentrations in the PN admixture of 0.4 mg/mL (≈2.35 mmol/L), 1.6 mg/mL (≈9.40 mmol/L) and 4.8 mg/mL (≈28.20 mmol/L). Stability and compatibility tests were performed over seven days at 0, 24, 48, 72 and 168 hours after admixing LEV injection solution into a PN sample. To prepare the test samples, the bag compartment seals were broken by mechanical pressing on the PN container. The bag was turned upside down five times for homogenization. Three samples (5 mL of PN) of each homogenized PN were transferred into 10 mL glass tubes using a 5 mL manual pipette. LEV injection solution was dosed by admixing 20 µL, 80 µL or 240 µL to the PN sample aliquots in the glass tubes, using manual pipettes. All pipettes in the range of 20-10’000 µL used had to pass the lab-internal testing within a specified accuracy of ± 1.0% and a coefficient of variation (CV) of ± 0.50%. The glass tube samples were covered with an airtight seal to provide an air- and fluid-tight seal. The test samples (PN with LEV) were stored in a refrigerator at +4 °C; at room temperature (23 ± 1 °C) in the laboratory; or at +37 °C (water bath). The samples were stored in transparent normal glass tubes without additional light protection. Samples stored and handled in the laboratory at room temperature or in the water

bath at elevated temperatures were exposed to daylight or artificial light (practical condition); samples stored in the refrigerator were protected from light by the closed door (“in the dark”). At each test time point, the samples in the tubes were visually inspected for discolorations, creaming, phase separation, or precipitates. Before taking an analytical sample, the tubes were turned upside down and back three times to guarantee a homogenous admixture. Three aliquots of each sample were measured by LC-MS/MS and then statistically calculated for drug concentration (mean ± SD for each sample). For the identification and quantification of LEV, a Thermo Fischer Ultimate 3000 UHPLC system (Thermo Fisher, San Jose, CA, USA) coupled to an ABSciex 4500 quadrupole mass spectrometer (ABSciex, Darmstadt, Germany) was used with a commercial kit (AED MassTox panel, Chromsystems, München, Germany). Mobile phases I and II were from Chromsystems, München, Germany [14]. The MS was run in the multiple reactions monitoring (MRM) mode using two transitions for each analyte. The Turbo V ion source run in positive ESI mode. Preparation and clean-up of the samples was performed according to the manufacturer`s protocol for serum samples [15]. Matrix effects and extraction recoveries were determined for serum and PN, as proposed by Matuszewski et. al.[16]. Three aliquots of all three concentrations were used. Thereby, the stock solution was diluted 1+1 (1:2) and also 1+99 (1:100). Sample aliquots of 50 µL were put into Eppendorf tubes, mixed with 25 µL of extraction buffer, vortexed for 10 seconds and then incubated for two minutes at room temperature. The internal standard mix (250 µL, containing precipitation reagent) was added, vortexed for 30 seconds and then centrifuged for five minutes at 13’000 rpm. Ten µL of the supernatant was mixed with 1090 µL of dilution buffer. The injection volume was 10 µL, and the LC flow rate was 0.6 mL/min. The injection was done by a thermo-controlled autosampler at room temperature (Thermo Scientific, Dionex UltiMate 3000), and the quantification was done according to a calibration curve, normalized to the corresponding internal standard.

Light microscope investigations for lipid droplet assessment and pH measurements (Metrohm 744 pH Meter) were performed for NOS and NLS as blanks and with concentrations of 1.6 mg/mL LEV (9.40 mmol/L) at +4 °C, +23 ± 1

°C and +37 °C. Before each pH measurement, a two-point calibration of the pH meter was done, each with a buffer solution of pH 9.00 and pH 4.00, respectively (Metrohm calibration buffer). The pH 7.00 solution was used afterwards as a control. Between the calibration steps, the electrode was rinsed with distilled water and wiped dry.

The physical stability of lipid emulsion was assessed by lipid droplet measuring in a light microscope (BX51 Olympus) with an upper droplet size of ≥ 1 µm. Each microscopic sample (10 µL by a manual pipette) was analysed with 100-fold magnification and oil immersion [17]. Five individual visual fields were inspected per microscopic sample (15 total visual fields/aliquot): four in the corner and one in the middle of the preparation. The size of the lipid droplets in the visual field was determined using an ocular micrometre (0.01 mm). The diameter of the largest lipid droplet (LLD) and the number of lipid droplets > 5 µm were measured and counted in each of the 15 visual fields tested per aliquot. The stability-indicating data were measured and calculated according to Schmutz (thesis). The specifications [17] of microscopic screening are shown in Table 2.

The statistical assessment was performed with IBM SPSS Statistics for Windows, Version 19.0 (IBM Corp. Released 2010, Armonk, NY, USA) and Prism 6 for Mac OS X, Version 6.0 (GraphPad Software). The results are reported as the means with standard deviations (mean ± SD) or as numbers and percentages (n, %). In addition, 95% confidence intervals, R^2, two-tailed P test, and one/two-way ANOVA were calculated. A *p*-value <0.05 was considered statistically significant.

Results

Microscopic analysis

The microscopic results of NOS and NLS with LEV (1.6 mg/mL) are shown in Table 3. The mean of the largest lipid droplet in µm out of 15 visual fields (n) ($MLLD_{max}$) of 4.5 µm as the upper limit value for the emulsion stability was never reached by any sample over the seven-day analysis period. The mean value of the $MLLD_{max}$ for NOS (at all temperatures and throughout the storage duration) was 2.3 ± 0.60 µm (n=180) and for NLS was 2.6 ± 0.63 µm (n=180). There was a trend for the droplet size to increase over time, as shown in Figure 2. The upper limit specification (specifications shown in Table 2) of a LLD in 15 visual fields of ≤ 8 µm was always reached. The LLD measured had a diameter of 8 µm (n=1). The highest SD was 1.99 µm and was thus always smaller than the 2.0 µm specification. Similarly, the number of lipid droplets > 5 µm was three was thus always smaller than the specification of nine.

The $MLLD_{max}$ of NOS and for NLS as blank samples at all temperatures and over the entire storage duration was 2.6 ± 0.77 and 2.7 ± 0.69, respectively, showing comparable emulsion characteristics, and the data were within the specifications. There was no statistically significant difference between the blank samples and the samples with added LEV (p=0.1602, two-way ANOVA). These specifications were not dependent on temperature (4-37 °C), and no difference of the different lipid compositions of NOS and NLS could be detected. The microscopic assessment of lipid droplets per sample (15 visual fields) took approximately 20 minutes.

pH determination

The pH of the different PN admixtures samples (with and without 1.6 mg/mL LEV) at three different temperatures decreased negligibly over time in NOS and NLS but ranged in all samples from 5.47 to 5.63 (mean ± SD: 5.55 ± 0.04, n=48), with no detectable changes during the test period. There was no difference regarding the different lipid compositions, and no temperature dependency was shown.

Visual inspection

Over seven days, no visual changes were observed in the test samples stored at +4 °C or at room temperature. There was no creaming or discoloration, except for the samples stored at +37 °C which showed yellowish discolorations after 96 hours. However, neither precipitates nor flocculation were visible. A visual inspection was done for the assessment of large particle formation in the critical size 1-5 µm.

LC-MS/MS analysis

Extraction recoveries and matrix effects for PN and serum were tested for two LEV concentrations. Serum analysis is described because this lab method was validated for therapeutic drug monitoring (TDM) in serum samples and has therefore to be checked when alternative samples were measured. Since the PN admixtures samples used produced no matrix effects, the analysis could be directly used for the stability measurements in NOS and NLS. The extraction recovery for PN samples for the lower concentration (0.4 mg/mL) was 98.7% (n=5, SD=5.53) resp. 86.0% (n=5, SD=5.77) for the higher concentration (4.8 mg/mL). The recovery rate for serum samples was 99% (n=5, SD=5.01) resp. 92.7% (n=5, SD=6.35). No matrix effects were observed: the matrix effect for PN samples ranged from 90.9-101.3% and from 97.2 -105.1% for serum samples.

No differences between NOS and NLS (as blanks) were observed for selected incubation/storage temperatures (one-way ANOVA: F=1.357, p=0.2655, R^2=0.0677). The three different LEV concentrations in NLS at +37 °C measured over 7 days were 0.43 ± 0.02 mg/mL, 1.76 ± 0.07 mg/mL, and 5.07 ± 0.15 mg/mL respectively. At room temperature, the following LEV concentration over time resulted: 0.43 ± 0.01 mg/mL, 1.83 ± 0.11 mg/mL resp. 5.11 ± 0.23 mg/mL . No storage temperature dependency was detectable, when comparing room temperature vs. +37 °C (two-tailed t-Test, p=0.3078). Also no significant difference was shown regarding concentration dependency (one-way ANOVA:

F=3.084, p=0.0643, R2=0.2044). The measured LEV concentrations in NOS at +37 °C were 0.43 ± 0.02 mg/mL, 1.72 ± 0.07 mg/mL resp. 4.96 ± 0.21 mg/mL. The measurements in NOS at room temperature resulted in 0.41 ± 0.01 mg/mL, 1.8±0.05 mg/mL resp. 5.25 ± 0.28 mg/mL. There was no significant change of the LEV concentration detectable over seven days when comparing the data at RT and +37 °C (two-tailed t-Test, p=0.2029). Additionally, there was no significant concentration dependency detectable (one-way ANOVA: F=3.037, p=0.0646, R^2 =0.1836). All results were in the range of ± 20%. The imprecision was within ± 20%. As the LEV measurements were done in highly complex o/w PN samples comparable to bioanalytical samples, the 20% range for deviation was applied. Although the best fit line showed negative trend over the storage time (y=-0.0259x+108.7, R^2=0.0847), no significant decline of the concentration resulted from one-way ANOVA: F=2.124, p=0.1076, R^2=0.2537. The individual dot in the stability over time plot indicates the mean of the three measurements expressed in % of initial concentration of LEV in NOS at room temperature and at +37 °C. (Figure 3).

Discussion

Although this stability assessment procedure is aimed for a more general approach how to evaluate on a short term base drug-PN stability and compatibility request in a hospital setting with easy to realise lab investigations available in teaching hospitals and pharmacies, it is important to validate the approach by appropriate investigations on representative and relevant drug products. In this study we selected LEV (Figure 1), a highly water soluble (> 100g/100 mL of water) low molecular pyrrolidine anticonvulsant representative. This amid drug has a pKa of -2 and is almost not protein-bound. Therefore, it represents a very hydrophilic (logP of -0.6), neutral not dissociated drug compound used therapeutically in molar doses (1000 mg ~ 6 moles) [13,18]. A review showed that anticonvulsants are often used together with PN in traumatic brain injuries and in cancer patients [19]. Stability and compatibility data with PN are scare, although it is a relevant and frequently used drug for critically ill patients or neonates. Medications often describe Y-site administration and do not address the chemical stability, which is important for efficacy and safety.

LEV in a dose range of 0.4-4.8 mg/mL (2.35-28.20 mmol/L) is a highly dosed i.v. drug and may thus have compatibility concerns. There was no significant decrease over seven days in NOS and NLS at three different temperatures. The assessment of the chemical stability of LEV was done using a validated stability-indicating LC-MS/MS quantification method that is also used for TDM. LC-MS/MS investigations involve highly sensitive, state-of-the-art identification and quantification methods with drug-specific detection available in most tertiary hospital medical labs (see chromatogram of LEV in Figure 4). The advantages of the LC-MS/MS method vs. HPLC-UV or –FLD (with fluorescence detector) - are the most simple and fast sample preparation, substantially less interference e.g. by co-medications, a shorter run-time and consequently a higher sample throughput and selectivity - clearly predominate a potential lower precision. But using a (deuterated) internal standards, a complete and good precision through all clean-up steps of the analysis can be achieved. Therefore, and as the drug stability / compatibility evaluation in PN can rely on an existing validated and verified LC-MS/MS method used within the TDM-panel in the institutional routine

operation of a hospital, their use is within the scope of the investigation for relatively easy access methods to get stability indicating data.

The analysis of the matrix effect and the extraction recovery showed the applicability for PN samples and using the serum sample cleanup. The three different LEV concentrations chosen correspond to the usual LEV dosing from 2x 250 mg to 2x 1500 mg applied in usual PN volumes (administered daily). The lower level (250 mg) and upper level (3000 mg) concentrations admixed to 625 mL PN yielded a concentration of LEV of 0.4 mg/mL, 1.6 mg/mL and 4.8 mg/mL. Admixed into 1250 mL PN (for adults), this would result in a concentration of 0.2 mg/mL, 0.8 mg/mL and 2.4 mg/mL. In addition, it was shown that LEV could be dosed in half of the volume due to its high solubility.

All of the measured concentrations of LEV in NOS and NLS were in the range of ± 20% of the theoretical value. The different temperature (storage) conditions used for two different high drug concentrations showed no influence on the stability of LEV. The variability (higher concentration after storage) can be explained by pipetting errors during the sample preparation or by the dilution, which yields an incorrect 100% target value. The volume ratio for the PN sample and the drug admixture was ≥ 1:20 and was therefore sensitive to small pipetting errors. The accuracy of the pipettes is shown above.

The AiO PN admixtures that were chosen are representative treatments for patients; they require PN administration through a centrally placed i.v. catheter (osmolality >> 800 mosmol). The two products differ in the fatty acid (FA) composition of the fat emulsion (Table 1). These PN products are prefilled, stable, three-chamber formulations, that can readily be made and used in most patients. Commercially available lipid emulsions have a mean particle size of approximately 0.25-0.5 µm in diameter, which corresponds to chylomicrons. The growth of the lipid droplets into large fat globules could also block small blood vessel (> 5 µm), e.g., in the lung, and are dangerous formulations that should not be used in patients [5,20]. The light microscope method according to Schmutz et al. is highly sensitive and practicable, with a simple equipment and a conventional method validated by Photon Correlation Spectroscopy (PCS) and the Coulter^{R}

method. Using a microscope with a 100-fold magnification allows the detection of particles approximately 1 µm in size or enlarged emulsion particles up to 20 µm in size. Furthermore, other non-lipid globules (such as particulate matters or precipitations) can also be detected using this method. The method provides an easy, sensitive, cost-efficient, time-sparing, and convenient way to test the physical stability of a lipid emulsion in the critical droplet size to indicate destabilisation (large fat droplet assessment ≥ 1-2 µm), and it is suitable for drug incompatibility testing in AiO PN admixtures [17].

LEV did not affect the lipid droplet sizing in microscopy assay over at least 24 hours and only showed only a slightly increasing $MLLD_{max}$ over a week at elevated temperatures (Fig. 2,3). Because the chemical analysis with LC-MS/MS showed that LEV possessed good stability in all three concentrations, only the concentration of 1.6 mg/mL was analysed by microscope. No influence of the FA composition on lipid stability could be detected. A single *in vitro* study showed LEV stability over 91 days in Ora-Sweet (a syrup vehicle used to simplify the process of flavouring and sweetening) and Ora-Plus (suspension adjuvant) at two different temperatures [21]. Our results showed that $MLLD_{max}$ in each visual field over seven days at three different temperatures was 2.3 ± 0.60 µm for NOS resp. 2.6 ± 0.63 µm for NLS and did not differ from the PN without the drug. No trend for an increase in the mean droplet size was seen when 9.40 mmol/L LEV was added. All other parameters were according to the specs. This supports the position of using physicochemical drug characteristics as a first evaluation of the incompatibility risk or the critical emulsion deterioration potential. Nevertheless, a lab analysis for such complex pharmaceutical formulation is necessary to document the pharmaceutical appropriateness of such medication and needs pharmaceutical expertise in the NST.

The aim of the present study was to use simple and time-saving evaluation tests to assess physicochemical stability and compatibility of LEV admixed to two common commercial AiO PN admixtures. The investigation was able to demonstrate easy-to-administer tests and document the compatibility and stability of the drug in these PN products. Pharmacists could use such tests in daily practice upon the request of such data as a prerequisite for safe and efficacious

treatment advice and to prevent incorrect handling and medication errors [22]. Another factor indicating the stability of PN AiO admixtures is the pH. The pH decreases over time in PN admixtures because of the hydrolysis of fat triglycerides. Additional chemical reactions yielding base or acidic products also affect the pH. For the lipid stability and lecithin emulsifier, a pH range of 5-8 is necessary. The negatively charged surface (phosphate moiety) prevents the coalescence of the lipid globules [2]. A pH below 5.0 favours lipid instabilities [23]. In our study, there was only a slightly decreasing pH over time (most affected the samples at +37 °C) because of destabilisation, degradation, and polymerisation but not a LEV-specific reaction.

All of the presented data are based on tests that were done in 10 mL glass tubes, whereas in reality, the two solutions would be mixed in either a Y-line, or in the PN bag if the drug was added there. Previous studies have evaluated the compatibility between PN admixtures and medications in a static manner [24, 25] or by simulating Y-site administration [11]. This study with the aim to get a response on drug stability and compatibility quickly in a given design simulates “worst-case” conditions with a defined contact time between the PN admixtures and a given drug concentration (LEV) but also to check the stability for drug and PN at daylight and storage at room temperature and at +37 °C but only in glass containers. To transfer the data to other containers or medical device materials, further studies have to be done, especially if reports e.g. drug adsorption or absorption exist like for lipophilic drugs and some plastics like PVC. This is not the case for LEV.

A limitation of the study is the fact that these results are related to PN regimen without vitamins or trace elements and the need for (home) PN, but there is a low risk from the LEV characteristics that additional interactions with these micronutrients would occur (red-ox reactions). An additional limitation is that only one specialty of LEV and only two different PN were checked. However, again, there is limited evidence of major differences among originator and generic i.v. formulations, although generic drugs may potentially not have identical ingredients or pH differences. Other PN regimes might differ in composition, but with a hydrosoluble, almost neutral drug such as LEV, there is a low risk for major

incompatibility. In either case another series with different PN regimens could be done in a relatively short time. Additionally, the neonatal situation was not specifically assessed. Neonates often have very limited line access, individual PN regimes and lower medication dosage, and LEV is a candidate for such an admixture. Because we performed *in vitro* pharmaceutical analysis and no *in vivo* clinical assessment, it would be beneficial to assess this stable pharmaceutical formulation *in vivo*. The absence of data on *in vivo* effects is a limit of these stability assessments and would be valuable for common drugs. The aim of this study was to demonstrate how incompatibilities and, therefore, unsafe and ineffective admixtures can simply be tested via a pharmaceutical approach.

Conclusion

We investigated the compatibility and stability of LEV in two common, commercially available PN AiO admixtures in adults at conditions of usual handling in a hospital. LEV demonstrated compatibility and stability in NOS and NLS in a concentration of 1.6 mg/mL (9.40 mmol/L) at three different temperatures. In addition, these results illustrated a valuable approach for determining the pharmaceutical stability and compatibility of drugs with PN in practice. This timely and cost-effective pharmaceutical approach of documenting the quality of complex therapeutic regimens increases the convenience and avoids medical errors in the clinical setting. It is crucial to combine methods to obtain a robust analysis of the chemical and physical stability of such admixtures. The demonstrated example of LEV admixed to AiO PN is important in patient care, is representative of drugs that are hydrophilic-neutral or that have weak acidity, and documents pharmacists' support for critical medication treatments in hospital practice. This procedure can be easily applied in daily clinical practice to fulfill the demands of determining stability and compatibility of different drugs in PN mixtures, when co-administration with PN and medications cannot be avoided. In future studies, we will analyse drugs with varying physicochemical profiles and clinical importance to further validate the procedure.

Table 1: Product information of the composition of tested TPN (NOS and NLS), in 625 ml bags

ingredient	unit	NuTRIflex® Omega special	NuTRIflex® Lipid special
Volume glucose solution	*ml*	*250*	*250*
Glucose monohydrate	g	99.0	99.0
Sodium dihydrogen phosphate dihydrate	g	1.56	1.56
Zinc acetate dihydrate	mg	4.39	4.39
Volume fat emulsion	*mL*	*125*	*125*
Soya-bean oil	g	10.0	12.5
Medium-chain triglycerides	g	12.5	12.5
Omega-3 fatty acids	**g**	**2.5**	**-**
Monounsaturated fatty acids	**%**	**11.4**	**13**
Polyunsaturated fatty acids	**%**	**34.0**	**30.7**
Ratio Ω-3:Ω-6		**1:2.7**	**1:7**
Essential fatty acids	**%**	**31.7**	**30.7**
*Volume amino acid solution**	*mL*	*250*	*250*
Amino acid content	g	35.9	35
Nitrogen content	g	5	5
Carbohydrate content	g	90	90
Lipid content	g	25	25
Non-protein energy	kJ (kcal)	2505 (600)	2505 (600)
Total energy	kJ (kcal)	3090 (740)	3088 (738)
Osmolality	(mOsm/kg)	2170	2090
pH-value		5.0-6.0	5.0-6.0

* not detailed

Table 2: Specifications of microscopic i.v. fat emulsion stability screening in 15 visual fields [17]

Microscopic parameter	Abbreviation	Unit	Specification
Largest lipid droplet in 15 fields	LLD 1-15	[µm]	≤ 8
Mean LLD	$MLLD_{max}$	[µm]	< 4.5
Standard Deviation	SDLLD	[µm]	≤ 2.0
Number of lipid droplets >5µm	LD > 5 µm	[n]	≤ 9

Table 3: Emulsion stability assessed by microscopic analysis of LEV (1.6 mg/mL) admixed to NOS and NLS and stored at three different temperatures

PN		**NOS**			**NLS**		
temperature		**23±1 °C**	**+37 °C**	**+4 °C**	**23±1 °C**	**+37 °C**	**+4 °C**
parameter	**$MLLD_{max}$ [µm]**						
storage duration	0	1.5	1.2	1.8	1.7	2.3	1.5
	24	2.3	2.1	1.9	2.5	3.3	2.2
	96	3.3	2.4	2.9	2.4	3.3	3.1
	168	2.7	2.7	2.6	3.3	2.5	3.1
parameter	**LLD 1-15 [µm]**						
storage duration	0	3	3	4	5	4	3
	24	6	4	5	5	6	4
	96	6	4	6	5	5	6
	168	6	5	8	7	5	6
parameter	**SDLLD [µm]**						
storage duration	0	0.74	0.77	0.86	1.23	1.22	1.06
	24	1.68	1.06	1.03	0.99	1.53	1.08
	96	1.45	1.12	1.41	1.18	1.22	1.30
	168	1.45	0.90	1.99	1.53	1.46	1.33
parameter	**LD > 5 µm [n]**						
storage duration	0	0	0	0	0	0	2
	24	1	0	0	0	3	0
	96	2	0	1	0	0	1
	168	3	0	0	2	0	1

LEV: Levetiracetam; NOS: Nutriflex® Omega Special; NLS: Nutriflex® Lipid Special; RT: room temperature

Figure 1: Formula for Levetiracetam ($C_8H_{14}N_2O_2$; MG=170.2 g/mol; logP -0.6; pH 7.0) [13]

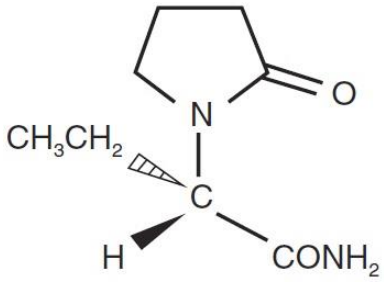


Figure 2: Mean values of the largest lipid droplet of NOS and NLS with LEV over time at three different temperatures

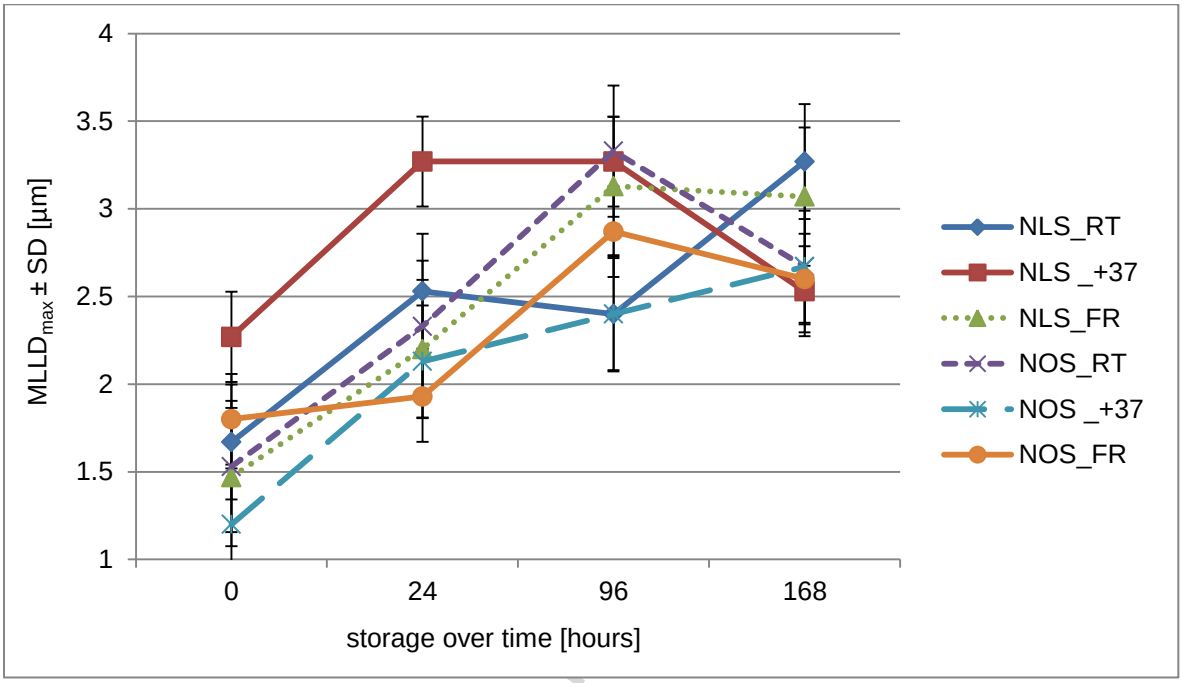


Figure 3: Stability of LEV in NOS over time determined by LC-MS/MS in mg/mL (room temperature and at +37 °C)

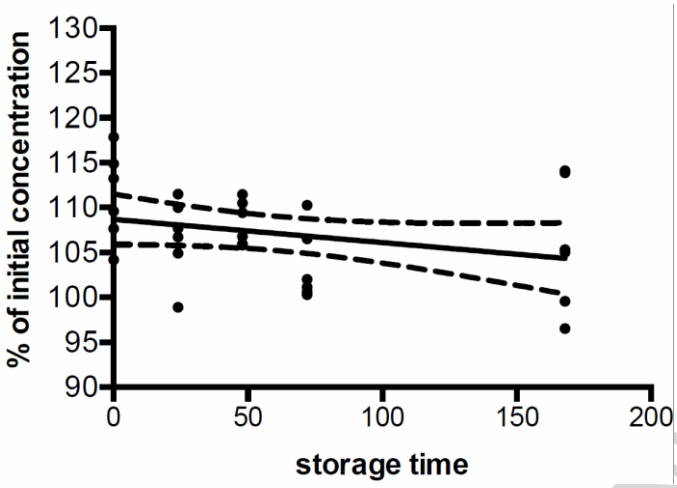
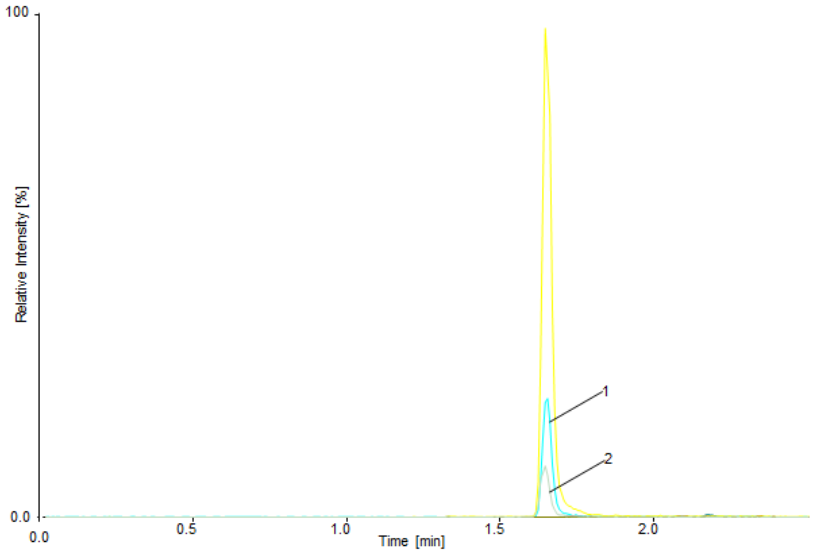


Figure 4: Extracted ion chromatogram (XIC) of Levetiracetam (1; yellow and cyan) and internal standard (2; grey).



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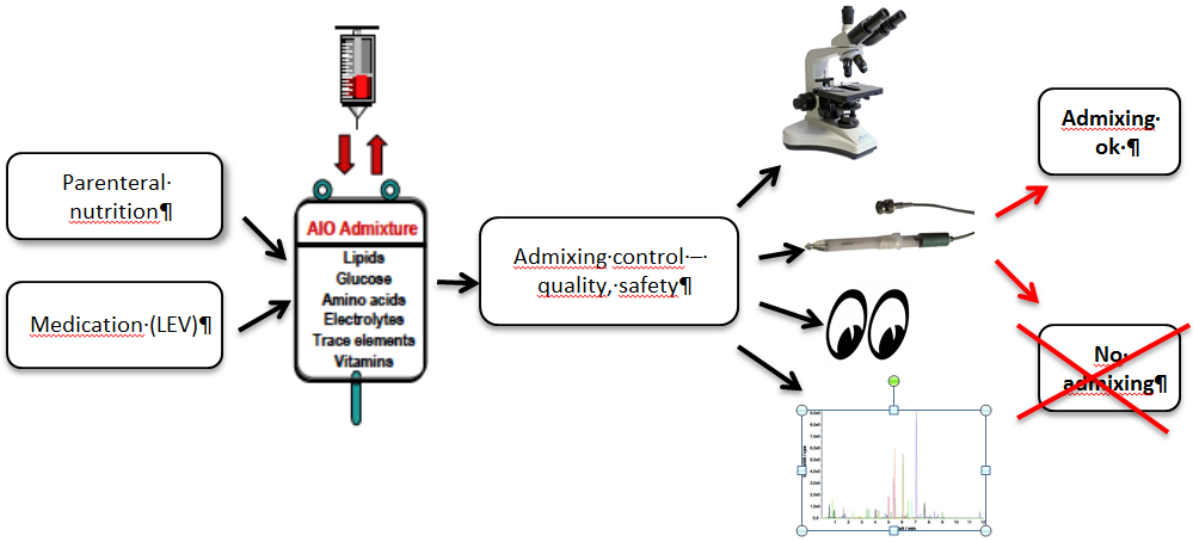

Graphical abstract